Report as of FY2008 for 2008TX308B: "Development of Library-Independent Bacterial Source Tracking Markers for Species-Specific Discrimination of Deer and Cattle Fecal Contamination in Surface Waters"

Publications

Project 2008TX308B has resulted in no reported publications as of FY2008.

Report Follows

Progress Report
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Title: Development of Library-Independent Bacterial Source Tracking Markers for Species-Specific Discrimination of Deer and Cattle Fecal Contamination in Surface Waters

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Abstract
Bacterial contamination is a significant cause of impairment and 303(d) listings of waterways in Texas. Efforts to track the source of fecal contamination have traditionally been conducted using labor-intensive library-dependent fingerprinting methods, for which geographical and temporal trends have yet to be determined. Library-independent methods utilizing indicator groups other than *E.coli* have been identified which do not require cultivation and are more cost and labor effective. Gut communities of anaerobic bacteria including *Bacteroides* have been shown to be host specific, and thus, amendable to the creation molecular markers specific to groups of warm-blooded animals. Markers specific to humans, dogs, swine and ruminants have been developed, but show the need for further validation. A lack of specific marker sets for relevant fecal contaminators limits their use today. The ruminant marker cannot distinguish between cattle and deer, but differentiating the two groups is especially important in Texas, as TMDL and best management practices are developed. To this end, the objective of this research is to develop molecular markers specific to a major wildlife faction in Texas, deer. These efforts will greatly enhance our ability to delineate three potentially key fecal contamination sources in Texas: cattle, humans, and wildlife.

Problem and Research Objectives
Culture-independent assessment of gastrointestinal flora of animals has indicated that *Bacteroides* strains makeup a substantial portion of the gut community and have thus become increasingly practical indicators of fecal pollution (7). *Bacteroides* sp. are strict anaerobes and have been shown not to persist in oxygenated waters (3), owing their existence in water to recent contamination events. Gut communities of anaerobic bacteria including *Bacteroides* have been shown to be host specific, and thus, amendable to the creation of markers specific to groups of animals (1). Molecular markers specific to humans, dogs, swine and ruminants have been developed, but show the need for further validation and a lack of specific marker sets for relevant fecal contaminators limits their use today (1). Notably, the ruminant marker does not discriminate between two important contributors, deer and cattle. The use of *Bacteroides* as a fecal indicator is a relatively young science, and expansion of our knowledge of this group of organisms will greatly enhance our ability to utilize them in bacterial source tracking projects.

The objective of this project is to develop deer specific *Bacteroides* molecular markers for use in bacterial source tracking. Deer constitute a considerable portion of wildlife in Texas. In rural parts of the state, deer are considered keystone species and as populations of both deer and people increase, so too will their interactions. Previous bacterial source tracking projects in Texas have implicated wildlife as a significant contributor to the fecal contamination load (2). Even though the *Bacteroides* cattle maker generally carries ruminant nomenclature, including deer, it has limited testing for ruminants other than cattle (1). And in Texas, separation of fecal contamination among ruminants, specifically between cattle and deer, is especially important as TMDL projects and best management practices are developed to alleviate bacterial impairments, mainly directed toward livestock management. Bacterial community analysis currently available in the literature was conducted on very small sample sizes and indicated vast sequence diversity in the *Bacteroides* community from different sources. These efforts suggested a more detailed analysis, including sequencing of *Bacteroides* community members, would hopefully allow for a better understanding of the host-specific microflora, and thus the development of specific markers for other groups (4, 5). Additional issues concerning wildlife identification in microbial source tracking projects arise when fecal sample collection strategies are examined. Collection of wildlife samples can be problematic as the animals are not easily accessible, but characterization of scat samples of unknown age and questionable origin should not be considered a sound practice when attempting to describe these communities (6).

Materials/Methodology

Fecal sampling was conducted at the Welder Wildlife Foundation, near Sinton, TX in January of 2009. A 2-6 inch section of the lower intestine was collected at time of deer kill and processed immediately. Five fresh cattle fecal samples were also obtained for use in comparative community analysis and molecular marker development. The samples were collected and stored on ice for cultivation studies or frozen on dry ice for genomic DNA extraction, and driven back to the Soil and Aquatic Microbiology Lab at Texas A&M University, College Station, TX within 36 hours. Fecal samples were frozen and stored at -80°C until DNA extraction. Fecal samples were also collected in the Leon River watershed near Comanche, TX during December and January 2009, and processed in the same manner. Both universal and ruminant *Bacteroides* primers will be tested with all of the samples to check for amplification with known primers following published protocols (1). A total of 12 fecal samples from both Welder Wildlife and Leon River watershed have been submitted for community analysis to Dr. Scott Dowd at Texas Tech University for 454 Sequencing. Sequencing of both Universal 16S rRNA genes as well as the general Bacteroides primer region are in progress. Sequences will be aligned and conserved regions of the sequences identified for the design of phylogenetic markers specific to the deer community. Validation of those newly created markers will be required. Fresh deer and cattle samples will be obtained from Welder again in the winter of 2009. In addition, other sampling locations in different geographical regions across the state may be selected to broaden the geographic scope of the project to aid in marker validation and community characterization.

Additionally, deer fecal slurries were used to isolate 10 *E.coli* isolates from each fecal sample from Welder to test against the Texas Bacterial Source Tracking library. Selected isolates have been ERIC-fingerprinted (2) and are in the process of being queried against the state library with the aim of adding isolate fingerprints to the ever-expanding library, and as collaborative data for the molecular marker design.

Expected Findings and Significance

This study will be the first of its kind to characterize the *Bacteroides* of a dominant member of the wildlife population in Texas. The community analysis alone will allow for the greater understanding of host populations of gut bacteria in this chief wildlife component. Cultivation-independent means of fecal identification though group specific molecular markers will be invaluable tools and serve as the next generation of microbial source tracking techniques to both quickly and confidently delineate fecal contamination. As molecular means of characterization become available and are validated, it will be imperative to combine new tools with current source tracking resources, including library-dependent *E.coli* based methods, to improve our ability to both track and prevent fecal contamination in an effort to tailor management practices and remediation schemes to ensure a healthy water supply.

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